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ORIGINAL PAPER

# Competition for S-containing amino acids between rhizosphere microorganisms and plant roots: the role of cysteine in plant S acquisition

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## Abstract

Plant S deficiency is common, but the role of S-containing amino acids such as cysteine in plant S uptake is unknown. We applied $^{14}$C-, $^{35}$S-, $^{13}$C-, and $^{15}$N-labelled cysteine to wheat and oilseed rape rhizospheres and traced the plants' elemental uptake. Both plants absorbed 0.37–0.81% of intact cysteine after 6 h with no further increase after 24 h. They absorbed 1.6–11.5% $^{35}$S and 12.3–7.6% $^{15}$N from cysteine after 24 h and utilised $SO_4^{2-}$ as their main S source (75.5–86.4%). Added and naturally occurring cysteine-S contributed 5.6 and 1.1% of total S uptake by wheat and oilseed rape, respectively. Cysteine and inorganic S derived from cysteine contributed 24.5 and 13.6% of uptake for wheat and oilseed rape, respectively, after 24 h. Oilseed rape absorbed ~10-fold more S from cysteine and $SO_4^{2-}$ than did wheat. The highest absorption of free cysteine should be in the organic-rich soil patches. Soil microorganisms rapidly decomposed cysteine ($t_{1/2}$ = 1.37 h), and roots absorbed mineralised inorganic N and S. After 15 min, 11.7–14.3% of the $^{35}$S-cysteine was retained in the microbial biomass, while 30.2–36.7% of the $SO_4^{2-}$ was released, suggesting that rapid microbial S immobilisation occurs after cysteine addition. Plants acquire N and S from cysteine via unidirectional soil-to-root nutrient flow, and cysteine is an important S source for plants.

**Keywords** Cysteine · Oilseed rape · Organic S · Plant S · Soil S cycling · Wheat

## Introduction

Sulphur (S) is an essential plant macronutrient. It plays a vital role in numerous metabolic processes such as coenzyme A, biotin, chlorophyll, glutathione, and thiamine biosynthesis (Wyngaard and Cabrera 2015). S deficiency in crops has been reported worldwide as a consequence of recent global reductions in atmospheric sulphur dioxide emissions, the use of low-S or S-free fertilisers, and soil S removal resulting from soil organic matter depletion (Dong et al. 2017; Wyngaard and Cabrera 2015). Organic S (OS) accounts for > 90–95% of all soil S (Kopittke et al. 2016). Nevertheless, most prior studies on plant S nutrition focused only on inorganic soil S and assumed that roots can only absorb inorganic sulphate (Ciaffi et al. 2013; Honsel et al. 2012; Prodhan et al. 2017). Hence, little research has been conducted on complex organic S as a plant S source (Bona and Monteiro 2010).

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Soil amino acids are produced by extracellular proteolytic enzymes, root exudation, soil microorganisms, and other processes. Several studies showed that plants absorb small-molecule organic N sources such as amino acids (Ganeteg et al. 2017; Hill et al. 2019b; Hill and Jones 2019; Ma et al. 2017) and quaternary ammonium (Warren 2013). Plants also absorb large-molecule organic N from sources such as nucleic acids and proteins (Paungfoolonhienne et al. 2008, 2010). Moreover, amino acids are highly bioavailable and have high turnover rates (Hill et al. 2011, 2019a; Jones et al. 2018a; Jones and Kielland 2012). Plants may absorb S-containing organic molecules such as cysteine and methionine and utilise the S they contain. However, amino acid content is substantially lower than the inorganic N content in the agricultural soil because of inorganic fertilisation, and amino acid-N plays a limited role in plant nutrition (Näsholm et al. 2009). When the inorganic sulphate content is low in the soil, soluble OS could be an important plant S source. Direct intact cysteine uptake could mitigate the energy expenditure required to convert sulphate to cysteine.

Plant roots must compete with soil microorganisms for amino acids. Soil microorganisms are the predominant soil organic matter consumers (Hill et al. 2013; Kuzyakov and Xu 2013; Näsholm et al. 2009). C and S from amino acids may rapidly undergo several complex reactions such as immobilisation into microbial biomass (MB) and the release of MB-bonded S as sulphate. Some of the intact amino acids and inorganic S derived from amino acids may be absorbed by plant roots (Ma et al. 2020a, 2021) (Fig. 1). The ability of soil microorganisms to decompose OS determines the amount of intact residual OS available to plant roots. Nevertheless, the connection between OS decomposition and root uptake remains to be established. Elucidating soil microbial cysteine decomposition will clarify soil C, N, and S cycles and their relationships with root macronutrient uptake.

Plant roots interact with soil microorganisms, and complex ecological and biological processes occur in the rhizosphere (Kuzyakov and Blagodatskaya 2015; Wei et al. 2019). Rhizodeposits affect microbial community function and composition (Liu et al. 2019) and promote fast-growing Gram-negative bacteria that can utilise low-molecular-weight organic matter (Dippold et al. 2014). Plant roots may face strong competition from soil microorganisms for organic N and S uptake in the rhizosphere.

To investigate plant OS uptake from the rhizosphere, we selected oilseed rape (*Brassica campestris* L.) and wheat (*Triticum aestivum* L.) for examination. These crop species markedly differ in terms of their S requirements. For optimum growth, wheat requires 15–20 kg S $ha^{-1}$, while oilseed rape needs 30–40 kg S $ha^{-1}$ (Vong et al. 2004). In the present study, we focused on cysteine, as it is the central metabolite coordinating S, C, and N flux in all chemoautotrophic and photoautotrophic organisms (Planta et al. 2017) and is usually present in various soil types (Cao et al. 2016). We performed $^{13}C$, $^{15}N$, $^{14}C$, and $^{35}S$ label uptake assays on wheat and oilseed rape cultivated in soil in rhizotubes. We used dual $^{13}C$ and $^{15}N$ labelling to distinguish intact amino acid absorption from the uptake of N derived from mineralised amino acids (Ganeteg et al. 2017). We also tested for linear correlations between $^{14}C$ and $^{35}S$ absorption. Radioisotopes are easily and precisely detected and measured. Moreover, foliar $^{14}CO_2$ release is simply and rapidly detected. Therefore, radiolabel assays were performed to evaluate the root cysteine-S/sulphate-S uptake ratios after mineralisation.

Soil available N content may also influence the competition for OS between roots and soil microorganisms by modulating microbial and plant element requirements. Hence, we explored how soil available N status affects plants utilising and microorganisms decomposing cysteine. We postulated that (1) roots have access to soil cysteine, but soil microorganisms strongly compete for it; (2) soil microorganisms rapidly decompose cysteine, and mineralised N and S may be available to plants; and (3) relative differences in plant S demand and soil available N content may reflect trends in plant preferences for organic and inorganic S.

## Materials and methods

### Plant uptake of intact OS from the rhizosphere

Agricultural brown earth soil (FAO classification: Eutric Cambisol) was sampled at a 0–10 cm depth at Henfaes Agricultural Research Station, Abergwyngregyn, Bangor, UK (53° 14′ N, 4° 01′ W). In the laboratory, vegetation, stones, and earthworms were removed from the samples, which were then air-dried to 20% water content and passed through a 2-mm sieve. Basic soil properties were determined using previously reported methods (Hill et al. 2013). The pH was 6.5; the total C and N concentrations were 34 mg $g^{-1}$ dry soil and 0.54 mg $g^{-1}$ dry soil, respectively; the total S content was 456 mg $kg^{-1}$; the soil contained 48.2% sand, 33.6% silt, and 18.2% clay; the soil solution amino acid concentration was 42 μM N; and the soil solution concentration of peptides < 1 kDa was 107 μM N.

Oilseed rape and wheat seeds were maintained in culture dishes for 2 days. Germinated seeds were individually sown in rhizotubes (length: 240 mm; internal diameter: 8 mm) containing 12.5 g agricultural brown earth soil (Fig. 1) (Hill et al. 2013). Aeration holes were cut into the rhizotubes at 1-cm intervals and served as injected labelling material ports. The plants were grown at 15 °C under a 16 h light/8 h dark photoperiod, 500 μmol $m^{-2}$ $s^{-1}$ light intensity, and 70% relative humidity for 17 days. Mean root dry weights were 24.9 ±

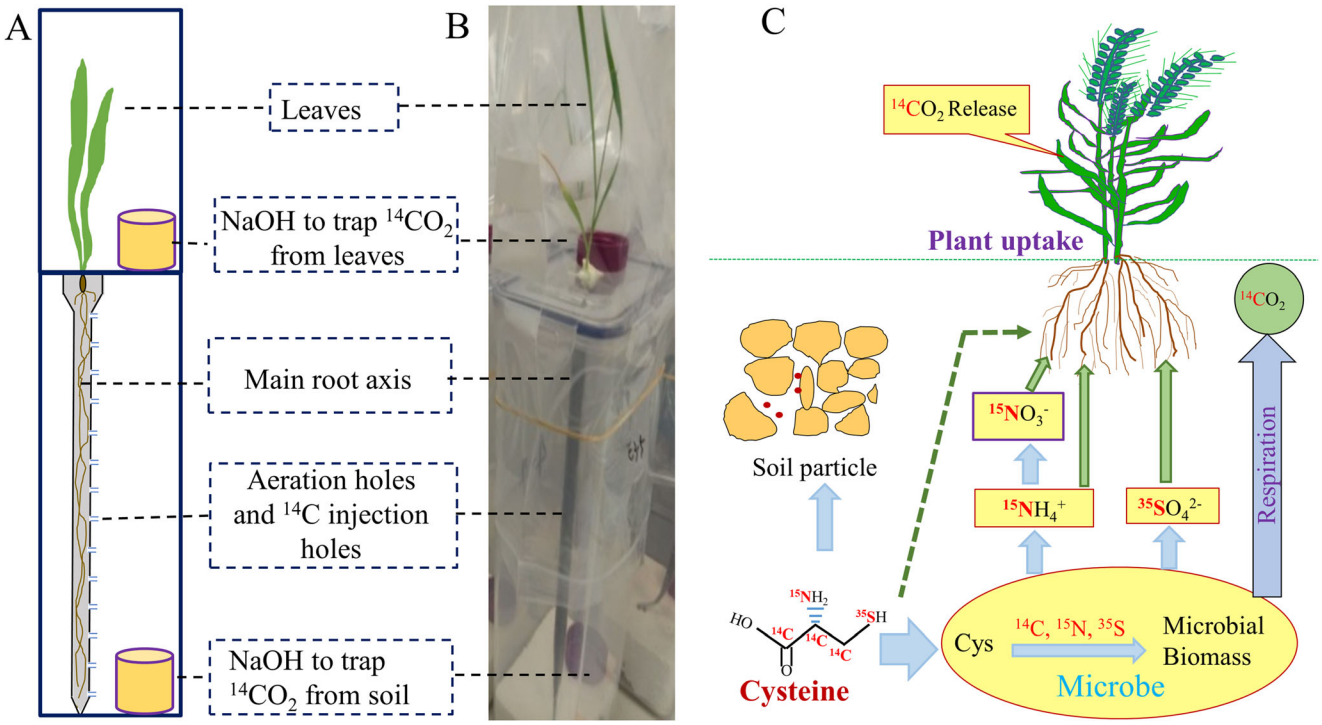


**Fig. 1** Schematic diagram (A) and photograph (B) of rhizosphere cultivation and $^{14}C$-tracing methods and simplified model of soil cysteine cycling (C). Wheat and oilseed were sown in rhizotubes with aeration holes. The holes also served as injected labelling material ports. The plants were cultivated for 17 days. Aliquots (1.2 mL) of the S treatments were injected into the rhizosphere at six equidistant points using 200 μL pipettes. Rhizotubes injected with $^{14}C$ were inserted into the apparatus, and their holes were sealed with silicone rubber to separate soil $^{14}CO_2$ and plant $^{14}CO_2$ release. The $^{14}CO_2$ was absorbed with 2 mL of 1 M NaOH. Possible dispositions of cysteine addition included (1) root uptake as intact molecules; (2) absorption of C in the organic S compound by the roots and its release from the leaves as $CO_2$; (3) immobilisation of C, N, and S in the microbial biomass; (4) release of $SO_4^{2-}$ and $NH_4^+$ by soil microorganisms; (5) absorption of $SO_4^{2-}$ and $NH_4^+$ by the plant roots; (6) release of microbial C as $CO_2$; or (7) adsorption of cysteine and its derivative $SO_4^{2-}$ to the soil particles

0.90 mg for wheat and 8.6 ± 0.4 mg for oilseed rape (n = 75). The wheat and oilseed rape roots filled the rhizotubes, and the soil around the roots was considered the rhizosphere. After 17 days, 1.2 mL of each S-treatment was injected at six equidistant points into the rhizosphere using a 200 μL pipette. In this way, the labelled materials could rapidly and uniformly disperse throughout the rhizotubes. We tested this method by adding the blue ink to the rhizotube in the same way as for the labelled solution, and all the soil in the tube turned blue in seconds. The injected solutions included 50 μM $^{35}S$-$^{13}C$-$^{15}N$-Cys, 50 μM $^{14}C$-Cys, 50 μM $^{35}S$-$^{13}C$-$^{15}N$-Cys + 15 mg N $kg^{-1}$ dry soil, 50 μM $^{14}C$-Cys + 15 mg N $kg^{-1}$ dry soil, and 50 μM $^{35}S$-$NaSO_4$ ($^{35}S$: 8.9 kBq $mL^{-1}$; $^{14}C$: 5.1 kBq $mL^{-1}$; L-$^{13}C_3$-$^{15}N$-Cys, 99.88%; Sigma-Aldrich Corp., St. Louis, MO, USA). Rhizotubes injected with $^{14}C$ were inserted into the apparatus shown in Fig. 1. The holes in the apparatus cap were sealed with silicone rubber to separate $^{14}CO_2$ released by the soil and plants. The $^{14}CO_2$ was absorbed using 2 mL of 1 M NaOH. The plants were destructively harvested after 15 min, 6 h, and 24 h uptake by vertically splitting the rhizotubes with a razor blade.

The soluble $SO_4^{2-}$ and cysteine concentrations were measured before the injection of the labelled solutions. Five grammes of rhizosphere soil was extracted for 30 min in 25 mL purified water in a flask on a reciprocal shaker at 20 °C and 180 rpm. The extracts were centrifuged at 12,000×*g* for 10 min at 20 °C, and the $SO_4^{2-}$ content was evaluated using ion chromatography (930 Compact IC Flex; Metrohm Ltd., Runcorn, UK). To measure the cysteine content in 5 mL supernatant, the protein was first precipitated with sulphosalicylic acid (Ozols 1990). The mixture was centrifuged at 5000×*g* at 20 °C for 5 min. The cysteine content was measured with an automatic amino acid analyser (L-8900; Hitachi, Chiyoda, Japan).

Five plants were harvested at each of the aforementioned time points (15 min, 6 h, and 24 h). The roots were separated from the soil by gentle shaking, washed with 0.01 M $CaCl_2$ for 1 min, and washed again with distilled water to remove any radiotracers on the root surfaces. The roots and shoots were separately harvested, freeze-dried (Labconco FreeZone Freeze-Dry System, Kansas City, MO, USA), and pulverised in a ball mill (MM301; Retsch GmbH, Haan, Germany). The $^{14}C$-labelled plant tissues were ashed in an OX400 biological oxidiser (Harvey Instruments Corp., Hillsdale, NJ, USA). Free $^{14}CO_2$ was captured with Oxosol scintillant (National Diagnostics, Atlanta, GA, USA), and the $^{14}C$ activity was measured by liquid scintillometry (Wallace EG&G, Milton Keynes, UK). The $^{35}S$ was extracted from 200 μg plant tissue

with 1.5 mL SOLUENE-350 (PerkinElmer, Waltham, MA, USA) for 24 h and centrifuged at 5000×*g* and 20 °C for 5 min. Then 0.4-mL extracts were mixed with 4 mL ScintiSafe 3 scintillation cocktail (Fisher Scientific, Loughborough, UK), and $^{35}S$ activity was detected with a Wallace 1404 liquid scintillation counter (Wallace EG&G, Milton Keynes, UK) (Jones et al. 2018a). C and N content and $^{13}C$ and $^{15}N$ incorporation in wheat and oilseed rape were determined with an elemental analysis-stable isotope mass spectrometer (IsoPrime100; Isoprime Ltd., Cheadle Hulme, UK).

The rhizosphere soil in a single rhizotube was divided into four 3 g portions. One portion was extracted with 15 mL of 0.01 M $CaCl_2$ ($^{35}S$-labelled) or 15 mL of 1 M KCl ($^{14}C$-labelled) to detect free labelled cysteine and produced $^{35}SO_4^{2-}$. The second was fumigated with 1 mL alcohol-free $CHCl_3$ for 24 h, and residual $CHCl_3$ was removed by vacuuming 5 five times for 50 min each time. The soil sample was extracted with 15 mL of 0.01 M $CaCl_2$ or 15 mL of 1 M KCl to detect $^{14}C$ and $^{35}S$ in the microbial biomass (Vong et al. 2004). The third was extracted five times with 50 mL of 1 M KCl to measure labelled cysteine and $SO_4^{2-}$ adsorbed to the soil particles (Cao et al. 2013). The moisture content of the fourth portion was determined by oven-drying the soil at 105 °C for 24 h. The solution was added to the soil samples and shaken at 180 rpm for 1 h. The samples were then centrifuged at 6000×*g* and 20 °C for 15 min. Then, either 0.5 mL purified water or 0.5 mL of 1 M $BaCl_2$ was added to 1 mL of the 0.01 M $CaCl_2$ extracts, and the mixtures were centrifuged at 18,000×*g* and 20 °C for 5 min. $^{35}S$ activity was detected, and the difference between samples was attributed to $SO_4^{2-}$ activity derived from labelled cysteine. The $BaCl_2$ precipitated the $SO_4^{2-}$ to $BaSO_4$ but had a negligible effect on the S-containing amino acids in the soil (Ma et al. 2020a, 2020b). The $^{14}C$ and $^{35}S$ activity levels were determined with a Wallace 1404 liquid scintillation counter (Wallace EG&G, Milton Keynes, UK) (Ma et al. 2020d). The $^{14}C$ and $^{35}S$ levels in the microbial biomass were determined by the fumigation-extraction method (Vong et al. 2004). The $^{14}C$ was extracted with 1 M KCl, and the $^{35}S$ was extracted from fumigated and unfumigated soil samples with 0.01 M $CaCl_2$. The $^{14}C$ and $^{35}S$ levels were measured as previously described. The MB-C and MB-S were calculated using the conversion factor 2.22 for C (Jenkinson et al. 2004) and 2.86 for S (Vong et al. 2004).

## Cysteine mineralisation in bulk soil and rhizosphere

Oilseed rape and wheat seeds were separately sown in rhizotubes containing 12.5 g agricultural brown earth soil and cultivated for 17 days. Sixteen rhizotubes were planted with oilseed rape, 16 were sown with maize, and another 16 contained only bulk soil. The soil samples in four tubes per treatment were combined into a single replicate. The water content and the environmental conditions were the same for all rhizotubes. Each 5-g soil sample was placed in a sterile 50-$cm^3$ polypropylene container to which 0.5 mL of 50 μM $^{14}C$-Cys, 200 μM $^{14}C$-Cys, 1000 μM $^{14}C$-Cys, 50 μM $^{14}C$-Cys + 15 mg $kg^{-1}$ N, or $^{14}C$-50 μM Cys + 1 mM $Na_2SO_4$ was added. The $^{14}C$ activity was 3.51 kBq $mL^{-1}$, N was supplied in the form of $NH_4NO_3$-N, and the $NH_4NO_3$ and $NaSO_4$ were dissolved in the cysteine solution. The $^{14}CO_2$ evolved from the soil was captured in a phial containing a 1 M NaOH trap and incubated at 20 °C. The $^{14}CO_2$ traps were replaced at 1, 3, 6, 9, 24, 48, and 96 h, and their $^{14}CO_2$ levels were determined by liquid scintillometry as previously described. To account for the $^{14}CO_2$ produced by the intrinsic microbial community in the $^{14}C$-labelled plant material (phyllosphere), control incubations were performed in the absence of soil (Jones et al. 2018b).

## Calculations and statistical analysis

The amounts of $^{13}C$ and $^{15}N$ derived from labelled cysteine and absorbed by wheat and oilseed rape were calculated by subtracting the quantities of $^{13}C$ and $^{15}N$ in the 'blank' seedlings from those in the treated seedlings (Sauheitl et al. 2009):

$$C_{uptake} = C_{total-C}(A_s - A_c) \quad (1)$$

where $C_{uptake}$ is the quantity of absorbed $^{13}C$ that originated in labelled cysteine, $C_{Total\text{-}C}$ is the total plant carbon, $A_s$ is the % of $^{13}C$ atoms in $^{13}C/^{15}N$-cysteine-treated wheat, and $A_c$ is the % of $^{13}C$ atoms in the 'blank' seedlings. The equation for $^{15}N$ uptake was similar to that used to calculate $^{13}C$ uptake. The ratio of plant $^{13}C$ and $^{15}N$ uptake was calculated by dividing the total $^{13}C$ addition by the total $^{15}N$ addition.

The ratio of $^{14}C$ uptake () by wheat and oilseed rape from labelled cysteine was calculated by subtracting the amount of $^{14}C$ in the 'blank' seedlings from that in the treated seedlings:

$$^{14}C_{\text{uptake ratio}} = (A_s - A_c)/^{14}C_{\text{total}} \quad (2)$$

where $A_s$ is the $^{14}C$ activity in the $^{14}C$-Cys treated plants, $A_c$ is the $^{14}C$ activity in the 'blank' seedlings, and $^{14}C_{Total}$ is the total $^{14}C$ activity added to the soil. The equation used to calculate $^{35}S$ was similar to that used to determine $^{14}C$.

The $^{35}S$ uptake after mineralisation ($^{35}S_{\text{uptake ratio-min}}$) was calculated as the $^{35}S$ uptake ratio ($^{35}S_{\text{uptake ratio}}$) minus the $^{14}C$ uptake ratio (intact cysteine uptake). This calculation was applied to each treatment as there were only five replicates. The linear relationship between $^{14}C$ and $^{35}S$ ($^{14}C = a^{35}S + b$) was shown and plotted as previously described by Ma et al. (2018).

$$^{35}S_{uptake\ \ ratio-min} = {}^{35}S_{uptake\ \ ratio} - {}^{14}C_{uptake\ \ ratio} \quad (3)$$

The soils contained some cysteine and $SO_4^{2-}$ in addition to the added labelled substrates. The amounts of N and S

absorbed were expressed in μM and derived from the quantity initially present in the original soil; the amounts added and calculated as follows:

$$S_{uptake} = {}^{35}S_{uptake\ \ ratio} \times 0.06 \times \frac{(\text{Content}_{soil} + 0.06)}{0.06} \quad (4)$$

where $S_{uptake}$ is the amount of absorbed S derived from cysteine or $SO_4^{2-}$, $Content_{soil}$ is the amount (μM) of soil-soluble cysteine or $SO_4^{2-}$ in a single pot (12.5 g soil; Table S1), and 0.06 is the amount of added cysteine or $SO_4^{2-}$ (0.06 μM).

The contributions of S (% of total S uptake) from intact or mineralised cysteine and $SO_4^{2-}$ were calculated as follows:

$$S_{\text{contibution}} = S_{\text{uptake}} / \left( S_{\text{uptake–Cys}} + S_{\text{uptake–SO}_4^{2-}} \right) \times 100 \quad (5)$$

where $S_{uptake\text{-}Cys}$ is the amount of absorbed S derived from cysteine (intact and inorganic S after mineralisation) and $S_{uptake\text{-}SO42-}$ is the amount of absorbed S derived from $SO_4^{2-}$.

Amino acid mineralisation was generally biphasic. Hence, it was described by a two-step, double first-order kinetic decay model (Glanville et al. 2016; Ma et al. 2020c):

$$f = \left(a_1 \times \exp^{-k_1 t}\right) + \left(a_2 \times \exp^{-k_2 t}\right) \quad (6)$$

where $f$ is the $^{14}C$ remaining in the soil, $a_1$ and $a_2$ are the quantities of $^{14}C$ partitioned between faster primary mineralisation (C $pool_1$; primary mineralisation; microbial respiration) and slower secondary mineralisation (C $pool_2$, biomass production) (Glanville et al. 2016), $k_1$ and $k_2$ are the exponential coefficients for $pool_1$ and $pool_2$, respectively, and $t$ is time.

The half-life ($t_{½}$) of $pool_1$ or $pool_2$ was calculated as follows:

$$t_{1/2} = \ln(2)/k_1 \quad (7)$$

The microbial C use efficiency (CUE) of the $^{14}C$-labelled substrates was calculated as follows (Glanville et al. 2016; Jones et al. 2018b):

$$\text{CUE} = \text{C}_{\text{pool 2}} / \left(\text{C}_{\text{pool 1}} + \text{C}_{\text{pool 2}}\right) \quad (8)$$

All data are presented as means ± SE. A Shapiro-Wilk test was used to assess normality before applying one-way ANOVA followed by Tukey's post hoc test ($P < 0.05$) to identify significant differences among treatments. The exponential decay equation was fitted to the experimental data in SigmaPlot v. 10.0 (SPSS Inc., Chicago, IL, USA). Graphs were plotted with Origin v. 8.1 (OriginLab Corp., Northampton, MA, USA).

## Results

The $^{14}C$ and $^{13}C$ assays showed that the plants absorbed 0.1–0.9% of the total added cysteine (Fig. 2). After 15 min, the wheat and oilseed rape absorbed 0.18 and 0.10% of the total $^{14}C$-cysteine, respectively. $^{13}C$ labelling indicated that they absorbed 0.15 and 0.38% of the cysteine, respectively. At 6 h, the $^{14}C$ and $^{13}C$ uptake levels nearly reached their maxima (wheat, $^{14}C$: 0.37 and $^{13}C$: 0.35; oilseed rape, $^{14}C$: 0.79 and $^{13}C$: 0.81). However, no further significant change in these values was observed at 24 h. In contrast, the $^{15}N$ and $^{35}S$ uptake rates markedly increased with time. At 24 h, the wheat and oilseed rape uptake levels ranged from 1.6 to 11.5% of the total added $^{35}S$ and 12.3 to 7.6% of the $^{15}N$. The oilseed rape and wheat absorbed similar amounts of $^{15}N$ at 24 h, while the oilseed rape absorbed ~6-fold more $^{35}S$ than did the wheat.

The $^{14}C$ and $^{13}C$ assays indicated that N addition did not affect intact cysteine uptake in the plants. Nevertheless, N addition increased wheat $^{35}S$ and $^{15}N$ uptake and decreased oilseed $^{35}S$ and $^{15}N$ uptake. Oilseed rape had a smaller biomass than did wheat but higher uptake ratios of intact cysteine and S derived from cysteine (Fig. 2). After $^{14}C$ was absorbed, 11.1, 31.3, and 54.1% of the total uptake were released as $^{14}CO_2$ from the wheat leaves, while 45.6, 29.5, and 69.3% of the total uptake were released as $^{14}CO_2$ from the oilseed rape leaves at 15 min, 6 h, and 24 h, respectively.

A linear relationship existed between $^{14}C$ and $^{35}S$ ($^{14}C = a^{35}S + b$) for wheat and oilseed rape. Here, 'a' represents the ratio of intact cysteine uptake to the total $^{35}S$ uptake. When a = 1, all absorbed $^{14}C$ is in the organic molecular form. The intact cysteine uptake ratios accounting for the total S uptake and based on $^{14}C$ and $^{35}S$ regression were 76.3, 64.3, and 22.8% for wheat and 82.3, 23.1, and 8.0% for oilseed rape at 15 min, 6 h, and 24 h, respectively. According to the $^{13}C$ and $^{15}N$ regression, however, the ratios were 14.2, 6.1, and 5.2% for wheat and 56.4, 17.3, and 11.2% for oilseed rape at 15 min, 6 h, and 24 h, respectively (Fig. 2).

After accounting for the soil cysteine and $SO_4^{2-}$ content, we established that wheat and oilseed rape use $SO_4^{2-}$ as their main S source. The uptake of inorganic S derived from cysteine increased over time. For wheat, the uptake of S from cysteine was higher than that from $SO_4^{2-}$ at 15 min. In contrast, oilseed rape utilised relatively more $SO_4^{2-}$ than cysteine after 15 min. N addition significantly enhanced cysteine-S utilisation by wheat but diminished its utilisation by oilseed rape after 24 h. S uptake from cysteine and $SO_4^{2-}$ was ~10-fold higher for oilseed rape than for wheat (Fig. 3).

Intact cysteine-S uptake accounted for 46.1% of the total S uptake by wheat but only 9.8% of the total S uptake by oilseed rape at 15 min. The contribution of intact cysteine-S declined to 5.6 and 1.1% for wheat and oilseed rape, respectively, after 24 h. At the same time, the contributions of intact cysteine and inorganic S derived from cysteine accounted for 24.5 and 13.6% of the total S uptake by wheat and oilseed rape, respectively. However, the contribution of $SO_4^{2-}$ accounted for 75.5 and 86.4% of the total S uptake by wheat and oilseed rape, respectively (Fig. 4).

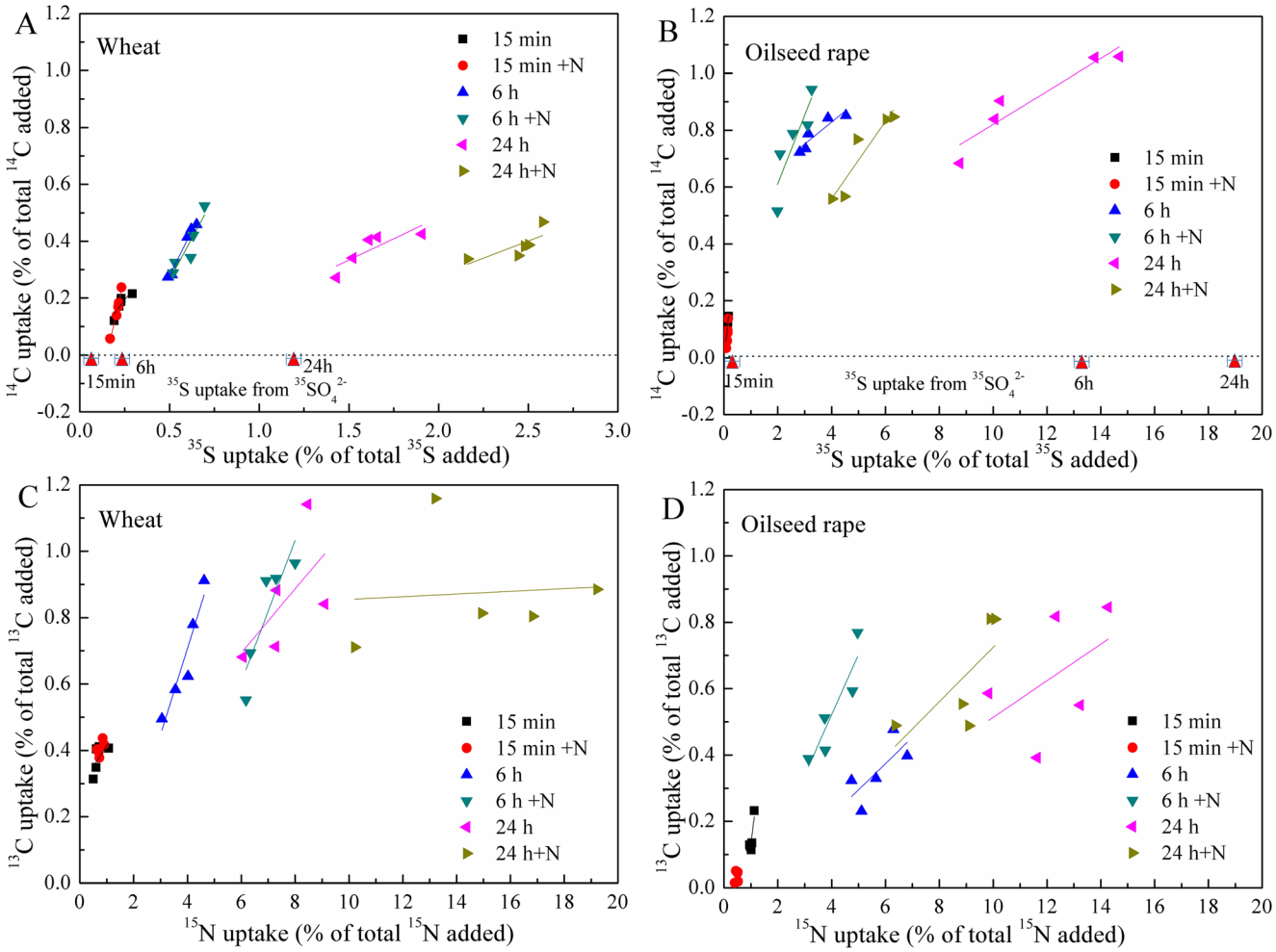


**Fig. 2** Uptake ratios of cysteine- and sulphate-derived $^{14}C/^{35}S$ and $^{13}C/^{15}N$ by wheat (**A**, **C**) and oilseed rape (**B**, **D**) in the rhizosphere at 15 min, 6 h, and 24 h

Fifteen minutes after cysteine addition, only 11.8–15.2% of the added cysteine remained in the soil solution, whereas 56.2–55.9% of the $^{14}C$ and 14.3–11.7% of the $^{35}S$ were retained in the microbial biomass (Fig. 5). After 24 h, the $^{14}C$ in the microbial biomass declined to 14.3–17.1%, while the $^{35}S$ in the microbial biomass rose to 19.0–28.8%. After 24 h, the amount of $^{14}CO_2$ released from the soil increased to 49.0–66.2%, whereas 52.7–54.5% of the total $^{35}S$-cysteine was released as $SO_4^{2-}$. At 15 min, 12.6–20.0% of the $^{14}C$-cysteine was adsorbed to soil particles, and only a trace amount was detected after 24 h. Almost half the added $SO_4^{2-}$ was retained in the soil solution, and 45.2–41.3% of it was retained in the microbial biomass by 24 h. N addition significantly increased the ratio of $^{14}C$ retained in the microbial biomass but had no effect on $^{35}S$-MB and decreased the $^{14}CO_2$ release ratio.

The $^{14}C$-cysteine decomposed rapidly in the soil ($t_{1/2}$ = 1.37 h). This rate did not substantially differ between the wheat and oilseed rape rhizospheres or the bulk soil (Fig. 6). N and S addition had negligible influences on the cysteine decomposition rate. However, soil cysteine decomposition decreased with increasing soil cysteine concentration. The $t_{1/2}$ were 2.79–2.85 h and 6.53–6.61 h for 200 μM cysteine and 1000 μM cysteine, respectively. Cysteine concentration and N and S addition had no apparent impact on C use efficiency (Table S2).

## Discussion

### Plant cysteine uptake from soil

In the rhizosphere, the roots compete with soil microorganisms for OS and ON. Microbes consume most soil organic matter, and plant roots can only access limited OS and ON (Hill et al. 2013; Kuzyakov and Xu 2013; Näsholm et al. 2009). In planted soils, amino acid turnover usually occurs within < 1 h (Farrell et al. 2014; Jones et al. 2009). Here, $t_{1/2}$ was 1.37 h for cysteine. The ability of soil microorganisms to decompose OS determines the amount of intact OS available

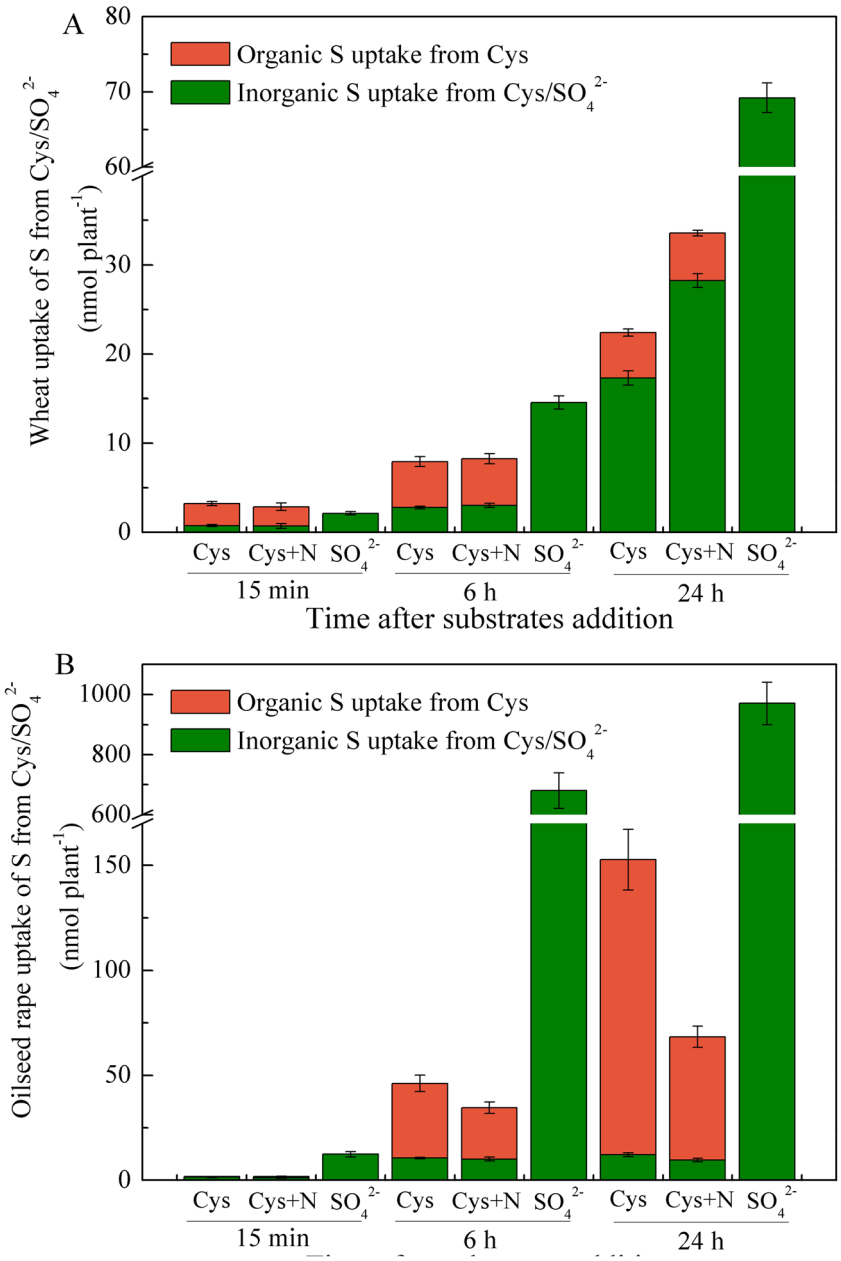


**Fig. 3** Uptake of S from cysteine (Cys) and sulphate by wheat (**A**) and oilseed rape (**B**) derived from added and native cysteine/$SO_4^{2-}$ in soil and calculated from $^{14}C$ and $^{35}S$ labelling at 15 min, 6 h, and 24 h. A portion of the inorganic S derived from cysteine mineralisation was included. Data are means ± SE of five replicates

to roots. Fifteen minutes after cysteine addition to the rhizosphere, only 11.8–15.2% of the $^{14}C$ remained in the soil solution, whereas 55.9–56.2% was retained in the microbial biomass, and 6.2–8.7% was mineralised to $^{14}CO_2$ by soil microorganisms. In contrast, the roots acquired only 0.10–0.18% of the total $^{14}C$ over the same time. The observed increases in $^{13}C$ and $^{14}C$ indicated that oilseed rape and wheat absorbed intact soil cysteine. When cysteine was mineralised, its C was transformed to $CO_2$, and plant roots could only access N and S. Plant cysteine uptake reached a maximum at 6 h. By 24 h, the soil cysteine had been depleted. This finding was consistent with the fact that negligible cysteine remained in the soil solution. However, most of the labelled cysteine-derived nutrients were utilised by the plants only after mineralisation to $^{15}NH_4^+$, $^{15}NO_3^-$, and $^{35}SO_4^{2-}$. Microorganisms outcompete plant roots for free amino acids in the soil solution. In the rhizosphere, microbial activity may be an order of magnitude higher than it is in bulk soil (Owen and Jones 2001). Hence, the roots had access to less cysteine than did the microorganisms (Ganeteg et al. 2017; Kuzyakov and Xu 2013; Ma et al. 2018).

Inorganic S and N liberated during microbial amino acid mineralisation are available as plant nutrients (Seegmüller and Rennenberg 2002). Here, cysteine rapidly decomposed to sulphate; 30.2–36.7% of the $^{35}S$ cysteine was converted to $SO_4^{2-}$ after 15 min, and the roots utilised the inorganic N and S. The microbial biomass absorbed the cysteine within 15 min, whereas plant uptake of cysteine-derived nutrients was far slower. The

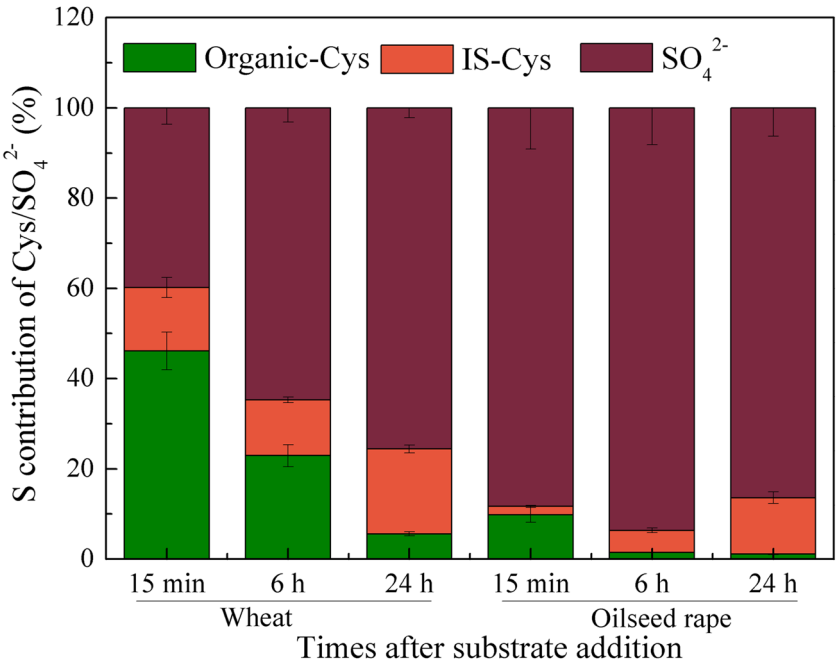


**Fig. 4** Contributions of S from organic amino acids and inorganic S derived from added and native $Cys/SO_4^{2-}$ in soil by wheat and oilseed rape as calculated from $^{14}C$ and $^{35}S$ labelling at 15 min, 6 h, and 24 h. Data are means ± SE of five replicates. Cys, cysteine; IS-Cys, inorganic sulphur derived from cysteine

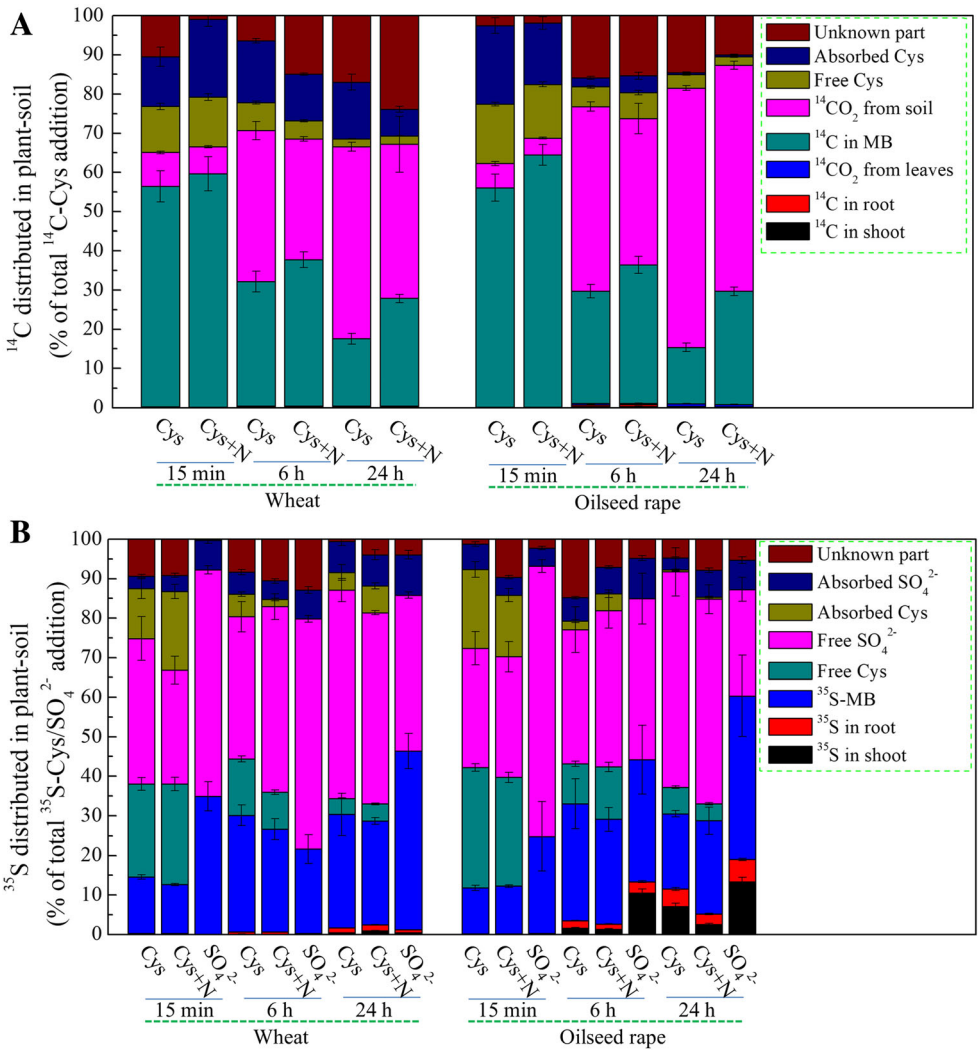


**Fig. 5** Distribution of $^{14}C$ (**A**) and $^{35}S$ (**B**) derived from labelled cysteine in plants and soil at 15 min, 6 h, and 24 h. Data are means ± SE; n = 5. Cys, cysteine; MB, microbial biomass

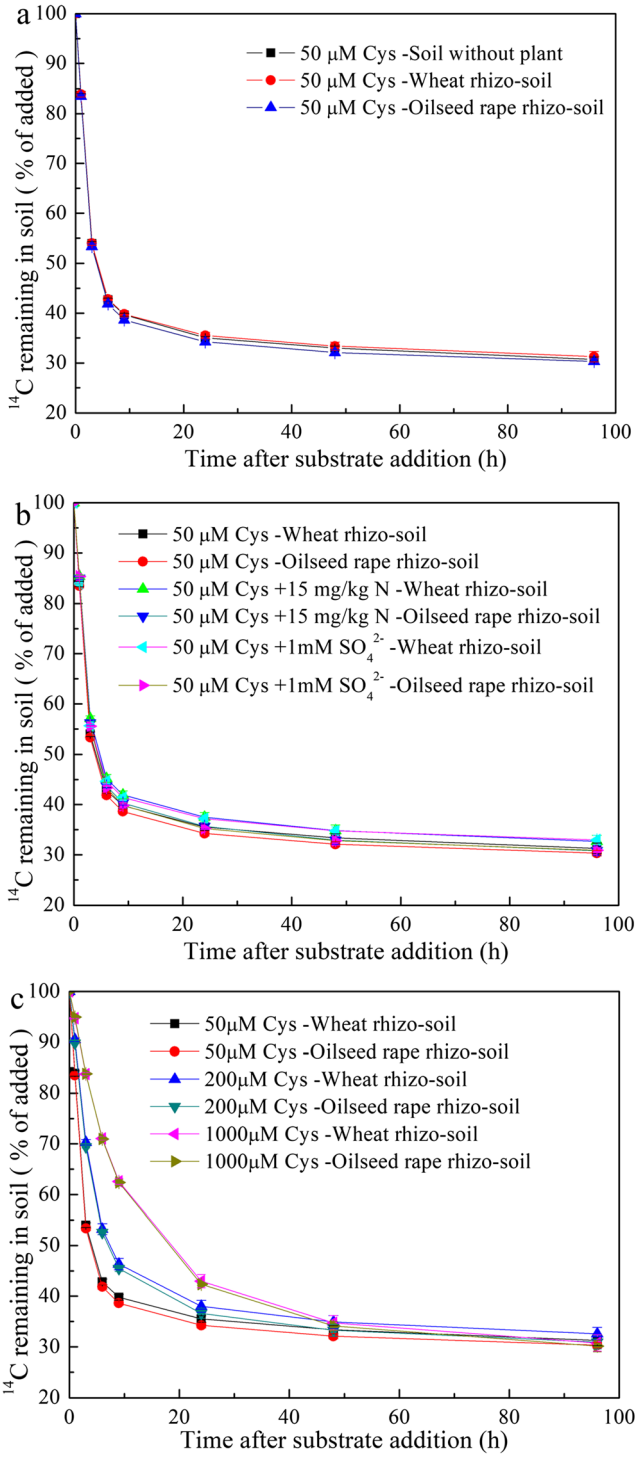


**Fig. 6** Effects of cysteine concentration and N and S addition on cysteine-C mineralisation in bulk and rhizosphere soils. Bars: means ± SE; n = 4. Cys: cysteine

latter process depended on the rates of microbial inorganic N and S liberation. After 24 h, wheat and oilseed rape absorbed 1.6–11.5% of $^{35}$S and 7.6–12.3% of $^{15}$N, respectively. Over the long term, then, plants prevail in terms of N and S acquisition as soil-to-root nutrient flow is unidirectional (Kuzyakov and Xu 2013). Despite strong competition for nutrients between plants and rhizosphere microorganisms, temporal niche differentiation induces mutualistic relationships that prevent N and S losses resulting from leaching during periods of minimal or no root uptake. Thus, roots receive a steady supply of available soil N and S (Kuzyakov and Xu 2013).

The rhizosphere experiments in this study demonstrated that wheat and oilseed rape differed in terms of their ability to utilise OS. Wheat roots used more intact cysteine than $SO_4^{2-}$, while oilseed rape absorbed 3-fold more S from $SO_4^{2-}$ than from cysteine after 15 min. Thus, oilseed rape might utilise $SO_4^{2-}$ as its main S source. In the soil, $SO_4^{2-}$ predominates in the highly bioavailable S pool. Moreover, oilseed rape efficiently utilises $SO_4^{2-}$ to meet its high S demand. Plants with coarse root systems (with various root exudates) such as oilseed rape harbour homogeneous microbial communities specialising in S immobilisation/remobilisation (Vong et al. 2002). Here, however, wheat and oilseed rape had minimal impact on root microbial OS decomposition. Hence, the differences between wheat and oilseed rape in terms of their capacity to utilise various S sources are determined by their individual root uptake characteristics rather than by microbial competition in the rhizosphere.

Approximately 46–72% of the $^{14}$C-cysteine absorbed by wheat roots was released from the leaves in the form of $CO_2$. In contrast, only 11–54% of the $^{14}$C-cysteine was released as $CO_2$ from oilseed rape leaves after 24 h. Hence, wheat has lower cysteine C-use efficiency than does oilseed rape, and the latter has a higher S demand than does the former (Scherer 2001). Wheat can immobilise more OS than oilseed rape, which might be due to its higher root biomass. Several studies used $^{13}$C/$^{15}$N dual-labelled organic N to measure the extent to which organic N meets plant N requirements (Ganeteg et al. 2017; Kuzyakov and Xu 2013; Ma et al. 2018). However, these studies did not consider foliar $^{13}$C release and may have underestimated the actual organic N contribution. Long-term OS uptake from the soil may have been underestimated because no other S-containing compounds such as glutathione and methionine were evaluated. Furthermore, cysteine was applied to the soil only by pulse addition and not continuously. In this study, the actual contribution might have been overestimated because we used a high cysteine concentration (Näsholm et al. 2009). Cysteine may undergo numerous changes in the soil, and a fraction of the $^{14}$C/$^{13}$C uptake from cysteine may be in the form of oxidation products such as cystine rather than intact cysteine per se.

## Soil cysteine-derived S, N, and C cycling

Elucidation of the mechanism by which microorganisms decompose organic S is crucial for the regulation and prediction of soil

S cycling. Cysteine decomposition by soil microorganisms involves rapid amino acid immobilisation into microbial biomass, the release of sulphate, and the reutilisation of the sulphate by soil microorganisms (Ma et al. 2020a, 2020b). Here, only 11.8–15.2% of the cysteine remained in the soil solution after 15 min. In contrast, 56.2–55.9% of the $^{14}C$ and 14.3–11.7% of the $^{35}S$ were retained in the microbial biomass, and 30.2–36.7% of the $SO_4^{2-}$ was released. Thus, the first and second steps (immobilisation into microbial biomass and $SO_4^{2-}$ release, respectively) may occur within minutes. In another study, 70% of the S originating from cysteine was retained in microbial biomass within 2 min (Ma et al. 2021a).

Rapid microbial S immobilisation predominates in S flux after cysteine is added to the soil. Individual amino acids may be removed from the soil solution (Czaban et al. 2016; Hill and Jones 2019) and mineralised to $CO_2$ and $SO_4^{2-}$ (Hill and Jones 2019; Wilkinson et al. 2014) within minutes to hours. The amino acids may serve as important N and S sources even at relatively low soil concentrations. The thiol group of cysteine is readily oxidised and is the key moiety in microbial and plant S cycling (Romero et al. 2014). Microorganisms may have mineralised the S without incorporating it, but relatively high C levels were retained in the microbial biomass. Furthermore, the microorganisms retained more of the C than the S originating from cysteine. Microbial OS mineralisation induced by microbial C demand could add to the existing plant S supply. After heterotrophs absorb the substrate, the N:C and S:C ratios may exceed certain thresholds leading to net N and S mineralisation, and extra N and S can be released as $SO_4^{2-}$ and $NH_4^+$ to maintain microbial biomass stoichiometry (Fan et al. 2020; Manzoni et al. 2017; Mooshammer et al. 2014; Wei et al. 2020). At 6 h and 24 h, $^{35}S$ MB increased, and the sulphate released was reutilised by soil microorganisms. Moreover, 12.6–20.0% of the $^{14}C$-cysteine was adsorbed to soil particles after 15 min. Part of this fraction might have been absorbed by the roots as the $^{14}C$ content had increased after 6 h (Cao et al. 2013). In addition, anaerobic processes, sulphate reduction and subsequent binding to minerals (such as FeS), and stable incorporation into microbial necromass might be responsible for the unknown part of this fraction.

## Factors influencing cysteine uptake

S and N assimilation are closely linked (Schneider et al. 2019). In the rhizosphere, N addition promoted wheat $^{15}N$ and $SO_4^{2-}$ uptake but inhibited both processes in oilseed rape. The differences between wheat and oilseed rape in terms of N and S uptake, transport, and demand might account for this discrepancy. Here, N addition had a negligible impact on cysteine decomposition by soil microorganisms, possibly because there was adequate N supply in the agricultural soil (Ma et al. 2020b).

Plant amino acid uptake reaches a maximum at high soil concentrations (Jones et al. 2005). Local soil amino acid levels after earthworm activity and clover decomposition can be as high as 45.3 mM and 2.7 mM, respectively, which suffice for root uptake (Hill et al. 2019a). High transient soil organic N and S concentrations occur after cell death. Soil $^{14}C$-cysteine decomposition is rapid ($t_{1/2}$ = 1.37 h), but this rate decreases with increasing cysteine concentration. For example, $t_{1/2}$ = 6.53–6.61 h for 1 mM cysteine. Plant cysteine uptake may reach a maximum at high concentrations wherein microbial cysteine utilisation is the lowest. Hence, plant OS uptake occurs mainly in organic-rich soil patches (Hill et al. 2019a; Jones et al. 2005).

## Conclusions

Wheat and oilseed rape can absorb intact cysteine from the rhizosphere even in the presence of soil microorganisms. The latter rapidly decompose cysteine, and the roots absorb mineralised N and S. Therefore, plants predominate in long-term N and S acquisition. Plant cysteine uptake reaches a maximum at high concentrations and occurs primarily in organic-rich soil patches. We conducted this research within agriculture soil, and the role of S-containing amino acids such as cysteine and methionine in plant S acquisition within natural soil requires further research.

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**Author contribution** QXM and DLJ designed research, conduct the experiments, and write the manuscript; PWH assisted in soil sampling, and PWH, DRC, and LHW revised the manuscript.

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## Declarations

**Conflict of interest** The authors declare no competing interests.

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